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Whipple's disease in a female with impaired cell-mediated immunity unresponsive to co-trimoxazole and levamisole therapy

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Summary

A case of Whipple's disease in a female is described. Malabsorption of iron, vitamin B_{12}, folic acid and fat was present. These abnormalities reverted to normal after introduction of co-trimoxazole therapy. The patient's humoral immune system was normal, in contrast to impairment of cell-mediated immunity, which has not improved in spite of co-trimoxazole treatment for 2 years, and a therapeutic trial of levamisole.

These findings are further evidence that there may be a primary immune deficiency in patients with Whipple's disease.

Introduction

Whipple's disease is a rare, systemic illness which usually affects middle-aged males. It is characterized morphologically by periodic acid-Schiff staining macrophages in virtually every organ system but maximally involving the lamina propria of the proximal small intestine and the mesenteric lymph nodes. In a recent review, 238 patients were reported as having Whipple's disease, of whom only thirty-three were female (Miksche *et al.*, 1974).

This report records the occurrence of Whipple's disease in a female with evidence of impaired cell-mediated immunity before treatment. Although clinical remission was induced with co-trimoxazole, evidence of impaired cellular immunity persisted. A therapeutic trial of levamisole for four months produced no significant improvement in her immune status. This lack of response supports the contention that primary impairment of cellular immunity is directly involved in the pathogenesis of Whipple's disease.

Immunological methods

Quantitative serum immunoglobulins and immunochemical serum complement levels were measured by single radial immunodiffusion. T-lymphocytes were estimated by their ability to form E rosettes with sheep red blood cells by the method of Jondal, Holm and Wigzell (1972). B-lymphocytes, i.e. cells bearing surface membrane immunoglobulin (SmIg), were quantified by immunofluorescence with anti-human Fab and anti-human μ chain antisera according to the method of Papamichail, Brown and Holborow (1971). SmIg and E rosettes are standard tests of B and T cell enumeration respectively. The whole blood culture method was used to quantitate lymphocyte transformation responses to mitogens and antigens (Paty and Hughes, 1972).

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Case report

A 54-year-old female presented in November 1970 with an 18-month history of joint pains responding symptomatically to phenylbutazone. This was a seronegative, non-erosive, arthropathy affecting the elbows, shoulders, neck and proximal interphalangeal joints of both hands, In March 1973, she developed abdominal pain, episodic diarrhoea and night sweats. Examination revealed anaemia, bilateral axillary lymphadenopathy and intermittent pyrexia up to 39°C. She was found to have a microcytic anaemia with a haemoglobin of 7·9 g/dl but a barium meal, barium enema and sigmoidoscopy were normal. Mantoux and Kveim tests were negative. She had steatorrhoea of 53 mmol/day (normal range <17) with positive faecal occult blood and abnormal xylose absorption. A barium follow-though examination showed a non-specific malabsorption pattern. Peroral jejunal biopsy was unsuccessful. A provisional diagnosis of coeliac disease was made and she was started on a trial of gluten-free diet.

In April 1974, she was transferred to the Metabolic Unit of the East Birmingham Hospital. She was cachexic, anaemic, with palmar erythema and bilateral axillary lymphadenopathy. There was abdominal distension, but rectal and sigmoidoscopic examinations were normal.

Investigations

Haematology

Haemoglobin, 11·7 g/dl; film normochromic

and normocytic; white blood cell count, $5{\cdot}1 \times 10^9$/l; direct lymphocyte count, $0{\cdot}78 \times 10^9$/l; ESR 50 mm fall/hr; serum iron, 2·86 μmol/l; iron binding capacity, 40 μmol/l; serum folate 2·2 μg/l; serum vitamin B_{12}, 418 ng/l; direct Coombs' test, negative. Bone marrow examination showed micro-normoblastic erythropoiesis with evidence of defective haemoglobinization, in the presence of a marked increase in iron stores.

Biochemistry

Urea and electrolytes normal; serum calcium, 1·98 mmol/l; serum phosphate, 1·23 mmol/l; serum magnesium, 0·74 mmol/l; alkaline phosphatase, 35 i.u./l; total serum proteins, 55 g/l; serum albumin, 27 g/l; serum orosomucoids, 2·0 g/l (normal range 0·30–1·20 g/l).

Immunology

Quantitative immunoglobulin estimations, lymphocyte transformation responses, and T and B cell estimations are shown in Tables 1 and 2. Antinuclear factor and Rose-Waaler test were negative. No serum cryoglobulins were detected.

Intestinal function

Faecal fat and nitrogen excretions averaged 123 and 214 mmol/day respectively during a 10-day balance period (normal range <17·4 and 71–142 mmol/day respectively). Iron absorption: following 100 mg of ferrous sulphate orally, the serum iron rose by only 3 μmol/l. Xylose absorption: 4% of the 5 g dose was excreted in the urine in the first 2 hours (normal range >15%) and 16% in the total 5-hour period (normal range 35±14%); the 1-hr blood xylose level was 0·62 mmol/l (normal range 0·65–1·33). A double isotope Schilling test showed malabsorption of vitamin B_{12} with and without intrinsic factor. Bromsulphthalein retention and urinary indican were normal. No pathogens were cultured from the stools and no ova, cysts or parasites were seen on microscopy.

Barium follow-through examination showed coarse mucosal folds in the duodenum and jejunum.

Culture of the jejunal aspirate grew *Escherichia coli, Clostridium welchii* and *Proteus mirabilis* but no ova, cysts or parasites were seen. Levels of trypsin, lipase and cholic acid in the fluid were normal.

She was initially considered to have a primary gastrointestinal lymphoma with a contaminated small bowel syndrome. The bacterial overgrowth was treated with co-trimoxazole for a period of 2 weeks and it was noted that her faecal fat excretion fell to a mean of 35·2 mmol/24 hr. A diagnostic laparotomy showed enlarged mesenteric lymph nodes and dilatation of the jejunum and ileum. The spleen measured only 10 cm along its longitudinal axis. A full thickness jejunal biopsy was taken 10 cm distal to the duodeno-jejunal junction and a mesenteric node excised.

Histology

The dissecting microscope appearance was of stout, swollen, finger-like or tongue-like villi. Under light microscopy (Fig. 1), the appearances were of Whipple's disease, with marked distension of the villi by accumulation in the lamina propria of many large histiocytes with abundant cytoplasm filled with PAS-positive granules. Immunofluoresence of the jejunal biopsy showed IgG deposition along the epithelial basement membrane and in the submucosa with increased numbers of IgA and IgM plasma cells around the base of the crypts. The histology of a mesenteric lymph node was also that of Whipple's disease, histiocytes containing PAS-positive granules and lipid-filled foam cells being present in abundance. Culture of the lymph node failed to grow any pathogenic organisms, including *Yersinia enterocolitica.*

Following the diagnosis of Whipple's disease, she was restarted on co-trimoxazole in standard dosage, plus folic acid supplements, with improvement in her clinical condition (Fig. 2).

Progress

Regular reassessment has shown clinical and biochemical improvement (Fig. 2). After 2 months of therapy, vitamin B_{12} absorption was normal. Reassessment at 1 year revealed elevated serum transaminases and HB_sAg was found to be positive in serum, saliva, jejunal juice, urine and faeces. Percutaneous liver biopsy was consistent with a residual stage of viral hepatitis and scattered cells were positive for HB_sAg both on orcein staining and using a HB_sAg antiserum in indirect immunofluorescence. Retrospective analysis of stored serum samples showed HB_sAg to have been absent at the time of diagnosis but present 3 months later.

She has continued on co-trimoxazole and was well when reviewed 18 months after diagnosis. A repeat jejunal biopsy was normal but cutaneous anergy to various antigens was still present and lymphocyte transformation responses to mitogens/antigens studied *in vitro* were mostly still impaired compared with controls (Table 1). The effect of the immunopotentiating agent levamisole was then investigated. The patient was given levamisole, 150 mg twice weekly for 2 months followed by 150 mg daily for 2 months. There was no fluctuation in her clinical state. Parameters of humoral and cell-mediated immunity were reassessed at the end of each 2-month period of levamisole therapy (Tables 1 and 2). The impairment of cellular immunity persisted despite this treatment.

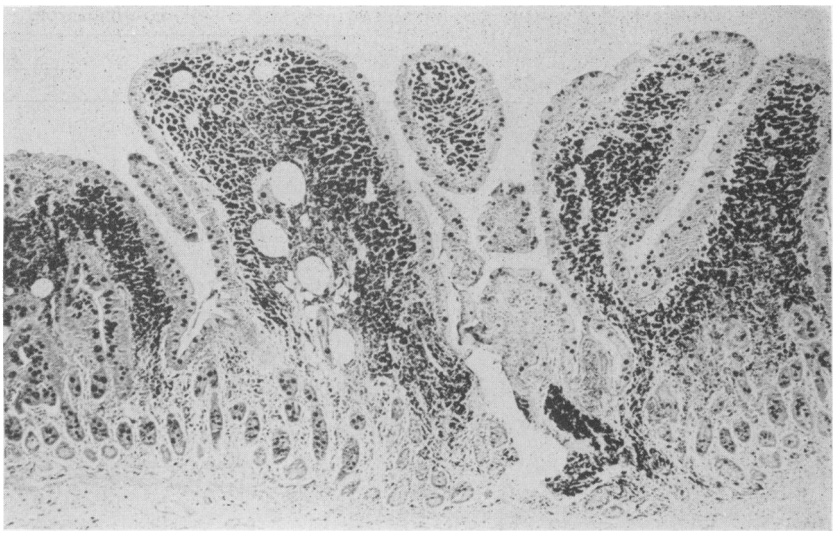

FIG. 1. Jejunal biopsy showing villi distended by characteristic PAS-staining macrophages in the lamina propria. (×77)

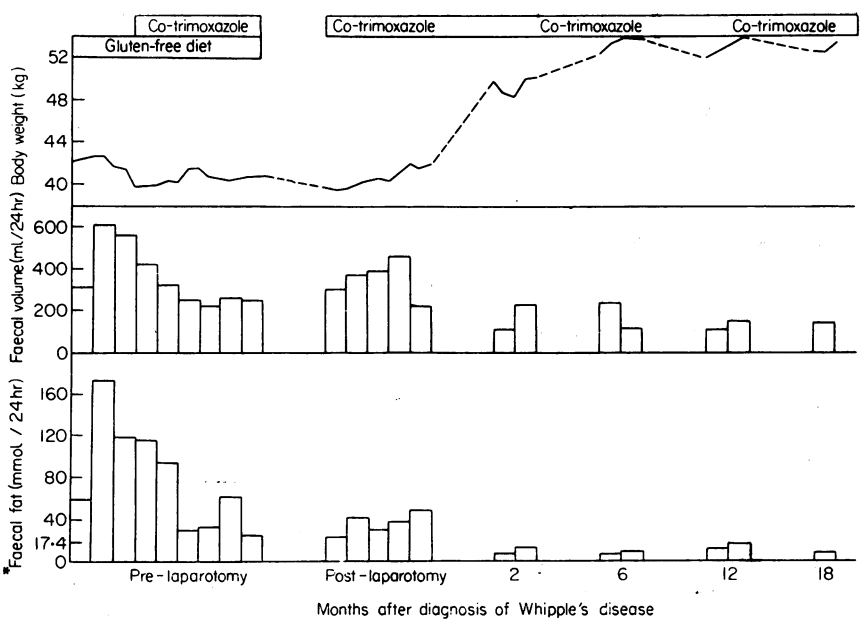

FIG. 2. Clinical response to co-trimoxazole. *Faecal fat results taken over a 5-day mean.

TABLE 1. Response of cell-mediated immune function to co-trimoxazole and levamisole therapy

Immunological studies (Values for control in parentheses)	Pre-treatment	Duration of therapy (months) co-trimoxazole			Levamisole		
		2	6	12	18	21	23
Lymphocyte transformation (counts/min × $10^{-2}/10^5$ lymphocytes)							
Unstimulated cultures	3·3 (2·2)	2·7 (3·6)	1·0 (1·2)	3·3 (4·8)	3·4 (1·4)	5·0 (6·7)	4·9 (6·8)
Phytohaemagglutinin 2·0 μg/ml	294·7 (448·1)	374·3 (345·0)	285·0 (437·0)	896·5 (1180·1)	131·4 (561·5)	106·6 (762·4)	157·8 (606·0)
Phytohaemagglutinin 0·2 μg/ml	50·4 (265·4)	86·7 (143·0)	76·7 (446·7)	627·5 (968·9)	56·5 (248·1)	69·8 (153·4)	58·8 (335·9)
Pokeweed mitogen	5·9 (130·5)	13·6 (40·6)	11·4 (64·8)	42·9 (126·8)	11·8 (74·3)	13·8 (109·4)	35·1 (167·1)
Purified protein derivative	2·2 (15·4)	4·6 (28·6)	8·0 (28·1)	4·1 (26·1)	10·1 (70·5)	2·5 (57·0)	7·7 (194·9)
Candida albicans	10·9 (35·8)	4·4 (17·9)	3·3 (13·9)	3·5 (21·4)	6·9 (38·4)	6·6 (12·6)	6·9 (16·5)
Tetanus toxoid	2·9 (2·9)	3·1 (2·6)	1·5 (3·2)	4·1 (3·5)	5·5 (1·8)	3·4 (6·9)	6·4 (5·6)
Cutaneous reactivity							
Purified protein derivative (10 tuberculin units)	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.	Neg.
Tetanus toxoid	Neg.	Neg.	—	Neg.	Neg.	Neg.	Neg.
C. albicans	Neg.	5 mm Induration	Neg.	Neg.	Neg.	Neg.	Neg.
Trichophyton	Neg.	Neg.	—	Neg.	Neg.	Neg.	Neg.

TABLE 2. Response of serum immunoglobulins, serum complement and lymphocyte sub-populations to co-trimoxazole and levamisole therapy

Immunological studies (Normal range in parentheses)	Pre-treatment	Duration of therapy in months co-trimoxazole			Levamisole		
		2	6	12	18	21	23
Quantitative serum immunoglobulins (g/l)							
IgG (6·0–16·0 g/l)	15·20	13·10	7·59	7·20	6·00	7·96	6·06
IgA (0·75–5·20 g/l)	3·58	2·95	2·03	1·90	2·52	2·06	2·06
IgM (0·30–1·80 g/l)	0·48	1·58	1·05	1·42	0·94	1·41	1·36
Immunochemical serum complement (g/l)							
C_3 (0·78–1·61 g/l)	1·27	1·39	0·95	1·39	1·25	1·38	1·31
C_4 (0·15–0·45 g/l)	0·39	0·25	0·19	0·23	0·18	0·20	0·21
Peripheral blood lymphocyte counts (cells/l)							
Total direct lymphocyte count (1·5–3·5 × 10^9/l)	0·78	2·24	2·03	1·60	2·35	1·45	2·32
% T lymphocytes							
(i) E rosettes (54–77%)	54	37	—	52	62	53	59
% B lymphycotes							
Surface immunofluorescence							
(i) Anti-Fab (9–21%)	—	24	—	20	21	18	—
(ii) Anti-IgM (4–7%)	—	9	—	20	3	4	—

Discussion

The arthropathy, recurrent pyrexia, peripheral lymphadenopathy and malabsorption present in this case are well documented features of Whipple's disease (Whipple, 1907; Maizel, Ruffin and Dobbins, 1970). The pathological findings in the jejunal biopsy and mesenteric lymph node fulfil the accepted criteria for its diagnosis (Maizel *et al.*, 1970). In the patient described, there was evidence of a functional defect in both the proximal small intestine (abnormal iron and xylose absorption) and distal small intestine (abnormal double-isotope Schilling test).

Only two other cases of significant malabsorption of vitamin B_{12} have been documented (Gross *et al.*, 1959; Paul, 1967), involvement of the distal small bowel being usually regarded as less severe than proximal disease (Maizel *et al.*, 1970).

Immunological studies of Whipple's disease have emphasized the cellular findings (Martin *et al.*, 1972). The significant lymphopenia initially present in the patient described affected both T and B lymphocyte sub-populations. This lymphopenia and the return to normal levels on treatment led Pastor and Geerken (1973) to suggest that, in untreated cases, there may be lymphatic obstruction with loss of lymph into the gastrointestinal tract. Untreated patients have generally shown depressed or absent responses to skin testing with a variety of antigens. In the case described pre-treatment cutaneous anergy was present and persisted despite otherwise successful therapy (Table 1). This is in agreement with the findings of Martin *et al.* (1972) but contrasts with other reports of partial or complete conversion of skin reactivity (Groll *et al.*, 1972; Pastor and Geerken, 1973). *In vitro* lymphocyte transformation responses to various mitogens and antigens were impaired both before and after treatment (Table 1), suggesting the defect was primary. This is supported by the fact that eleven of a total of thirteen patients studied have shown depressed responsiveness to phytohaemagglutinin in the post-treatment phase (Maxwell *et fal.*, 1968; Watson, Maxwell and Ferguson, 1969; Martin *et al.*, 1972; Groll *et al.*, 1972; Pastor and Geerken, 1973).

Where assessment of splenic size in Whipple's disease has been possible, the spleen was usually normal, although splenomegaly has been documented in at least six patients in the literature (Maizel *et al.*, 1970). The small spleen found at laparotomy in the present patient may be aetiologically related to the cellular deficit demonstrated, but no objective assessment of splenic function was made. The contined presence of HB_sAg despite clinical recovery and levamisole therapy could be taken as further evidence of defective cell-mediated immunity; patients with impaired T cell systems are known to be susceptible to viral diseases (Soothill, 1975).

With respect to humoral immunity, no clear-cut pattern of pre-treatment immunoglobulin levels has yet emerged. Groll *et al* (1972) demonstrated elevation of all immunoglobulin classes in their patients, but others have reported slight decreases in IgG with normal IgA and IgM levels (Case Records, 1971; Pastor and Geerken, 1973). Hypogammaglobulinaemia has been reported in four patients with Whipple's disease (Sandor and Kozmer, 1967; Martel and Hodges, 1959; Anton, 1961; Cochran *et al.*, 1973) and Berens, Cohen and Schwabe (1969) described a patient with decreased IgM levels. Post-treatment immunoglobulin levels have tended to be normal, with the exception of IgM, which has been variously reported as depressed (Maxwell *et al.*, 1968; Groll *et al.*, 1972) or elevated (Pastor and Geerken, 1973). The present patient had a high-normal pre-treatment IgG level which has fallen to a low-normal level on therapy, while IgA and IgM levels have always been normal (Table 2). Immunofluorescence of the jejunal biopsy showed increased numbers of plasma cells, particularly of IgA and IgM type, perhaps reflecting an increased mucosal response to local antigenic challenge. The serum complement activity, as reflected by serum C3 and C4 levels, has remained within the normal range before and after treatment.

Since Paulley (1952) reported the successful use of antibiotics, a variety of different drugs has been tried and found effective to varying degrees (Miksche *et al.*, 1974). Co-trimoxazole was initially used in the present patient to treat her contaminated small-bowel syndrome, but it also appears to have been effective in the treatment of the Whipple's disease (Fig. 2); Elsborg, Gravgaard and Jacobsen (1975) have also documented a case in whom this drug was used beneficially.

Any hypothesis of the aetiology of Whipple's disease must take into account its rarity, predilection for male Caucasians, predominant involvement of the gastrointestinal tract, and the histological observation of bacilliform bodies in the intestinal lesion. The true role of micro-organisms in this condition is not clearly defined but a cell-wall deficient form of an α-haemolytic streptococcus has recently been isolated from prolonged monolayer cultures of a lymph node taken from a patient with Whipple's disease (Clancy *et al.*, 1975). There is also some suggestion that host factors may be of prime importance. Evidence of impaired cell-mediated immunity in individuals with Whipple's disease (Maxwell *et al.*, 1968; Watson *et al.*, 1969; Martin *et al.*, 1972; Groll *et al.*, 1972; Pastor and Geerken, 1973) raises the possibility that a primary immune deficit predisposes to the development of the condition and the findings in the present patient support this suggestion.

Levamisole, an effective anthelmintic, has been shown to have immunopotentiation effects (Leading Article, 1975). In man, it has been reported as restoring delayed hypersensitivity responses in individuals with anergy associated with old age and malignant disease (Tripodi, Parks and Brugmans, 1973; Leading Article 1975). Clinical usefulness for levamisole has been claimed in a range of disorders (Leading Article, 1975) and *in vitro*, levamisole appears to activate functionally defective T cells (Verhaegen *et al.*, 1975; Biniaminov and Ramot,

1975). No significant improvement in the present patient's lymphocyte transformation responsiveness or cutaneous anergy was seen after 4 months of levamisole therapy (Table 2). This lack of response again suggests that the cellular defect in this patient was primary. Based on the evidence in the literature and the experience gained from the present case, it seems possible that impaired cell-mediated immunity is directly involved in the pathogenesis of Whipple's disease.

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